Summary

Study Title: "Evaluation of Experimental Dentifrice Formulations For Reducing Plaque and Gingivitis Using the Toothshield Clinical Model"

Clinical Study 03PGC-H2

Conducted at: University Park Research Center, Fort Wayne, Indiana
Principal Investigator: Dr. Mark S. Putt, MSD, PhD

Background:

Conventional long-term studies for establishing the gingival health effects of anti-plaque dentifrices are time-consuming and require large populations. Typically, such clinical trials last 6 months. Because of wide variations in the development of gingival inflammation between individuals, large or carefully selected populations are required in order to discriminate between treatments, thus making such studies expensive to conduct. Consequently, such studies do not usually permit the evaluation and comparison of more than two experimental products versus a control product. These time, cost, and sample constraints limit the use of traditional gingivitis clinical trials for developmental purposes.

A different approach, the experimental gingivitis model (EG) of Löe *et al.*, decreases the time required for the development of gingival inflammation by having subjects abstain from brushing their teeth for 21 days. By this abstention, subjects develop gingivitis more rapidly, which provides a shorter and more efficient means for evaluating therapeutic agents. However, the original EG study design presents difficulty in obtaining and retaining subjects for the 21-day study period due to the absence of toothbrushing of the whole mouth. Putt *et al* have proposed modifications to the original EG model to address these problems. Their modification involves promoting gingivitis in a specific region of the mouth (usually a mandibular quadrant) by covering this area during brushing with a tooth shield while allowing subjects to still brush the remaining three quadrants of their mouth. This procedure avoids much of the aesthetic unpleasantness resulting from cessation of brushing required in the original EG model.

Unlike the original EG model, it is proposed that this model can be used to assess the efficacy of all oral care forms. In the case of chemo-therapeutic toothpastes, both the direct chemical effects of the antimicrobial as well as those actions synergistic with brushing can be measured. This is accomplished by applying undiluted dentifrice directly to the cavity of the tooth shield and holding in place while the remainder of the dentition is brushed with therapeutic dentifrice. Consequently, treatment effects by the agent alone (under the tooth shield) and in combination with brushing can be determined.

To determine the utility of the tooth shield model for assessing dentifrice efficacy towards gingivitis, two different active ingredient systems incorporated into dentifrices were evaluated in this study. One contained 0.454% stannous fluoride and was formulated as Crest Gum Care, a commercial toothpaste, which has proven anti-caries and anti-gingivitis activities. This was compared to the antigingivitis activity of two experimental dentifrice formulations.

Objective

The main objective of the study was to evaluate the effects of commercial and experimental dentifrices containing different antimicrobial systems on dental plaque accumulation and prevention of gingivitis under accelerated conditions of plaque formation and gingivitis development using a 21-day, partial-mouth (tooth shield) gingivitis model.

Study Design Summary

This study was a comparison of parallel groups of subjects provided with either placebo or known/putative therapeutic dentifrice products using a short-term clinical model in which plaque formation and the development of gingivitis were facilitated in a mandibular quadrant of the mouth by use of a tooth shield.

The study was conducted in 2 phases. The first phase was a pre-trial hygienic period designed to reduce any existing plaque and gingivitis within subjects so that they would approach optimum oral health prior to initiating the trial (experimental gingivitis) period in the second phase. In the first phase tooth shields were constructed for a selected mandibular quadrant and custom-fitted. Gingivitis scores at the conclusion of the first phase were used to randomly assign subjects to equivalent groups for the trial period.

The second phase was a trial period of 21-days during which oral hygiene (tooth brushing) was suspended in either the left or right mandibular quadrant of the mouth which was covered by the fitted tooth shield. Subjects were instructed to brush all non-shielded teeth with a full brush head (approximately 1.5 g) of their assigned dentifrice for 1 minute twice daily while wearing the tooth shield whose cavity was filled with the same dentifrice (approximately 1.5 g). Following brushing and expectoration of the dentifrice slurry, subjects removed the tooth shield from the covered teeth and rinsed once with 15 mL of water for 10 sec. to remove any remaining toothpaste.

The following clinical assessments were made at baseline (beginning of Phase 2) and final (21 days of using tooth shield) for the following:

a) Gingivitis was measured separately for brushed teeth as well as those protected by the toothshield of the mandibular quadrant. Gingivitis status was measured by the use of the Modified Gingival Index for inflammation and the Gingival Bleeding Index. In addition, gingival status was assessed at the final visit using the Loe-Silness Gingival Index, which combines both inflammation and bleeding attributes into a single scale.

b) Plaque was measured separately for brushed teeth as well as those protected by the toothshield of the mandibular quadrant. Plaque status was measured by the use of the modified Quigley-Hein Plaque Index, and

c) Overall oral soft tissue health.

In addition to clinical assessments, dentifrice effects on bacterial accumulation along the buccal maxillary gumline were also determined.

Subjects were randomly assigned and stratified based on their pre-trial (baseline) gingivitis scores to one of 4 dentifrice treatment groups:

1) Experimental dentifrice placebo (served as negative control),
2) 0.454% stannous fluoride dentifrice formulated as Crest Gum Care (served as positive control),
3) Experimental dentifrice formulation A, and
4) Experimental dentifrice formulation B.

Each group was targeted to have approximately 35 evaluable subjects at the final visit.

Results

For all analyses, there were at least 35 evaluable subjects per treatment group.

Consistent with the intent of the model, clinical results for the negative control showed a increased development of gingivitis and plaque in those sites covered by a tooth shield for the 21-day experimental period compared to those sites that were brushed (shown below). Of the 2 clinical attributes of gingivitis, the lack of brushing caused by use of the tooth shield affected gingival bleeding more so than gingival inflammation.

Effect of Toothshield on Gingivitis and Plaque Development
(% change from baseline for Negative Control)

Index	Shielded Sites	Brushed Sites
MGI (gingival inflammation)	72	21
Gingival Bleeding	218	-13
Plaque	31	-5

Tables 1 & 2 show the treatment effects observed for the shielded sites across the various gingivitis indices employed in this study. When compared versus the negative control, the positive control stannous fluoride dentifrice provided significant ($p < .05$) reductions, ranging from 22 to 43%, across the various gingival indices. Similarly, the experimental dentifrice formulations A & B also provided significant ($p < .05$) reductions in gingivitis, ranging from 7 to 31%, across the various indices when compared to the negative control. Overall, the magnitude of anti-gingivitis benefits provided by the experimental dentifrices were no different from those achieved with the stannous fluoride dentifrice.

The stannous fluoride and 2 experimental dentifrices also significantly ($p < .05$) reduced plaque coverage when compared to the negative control in both the shielded and brushed sites with the magnitude of reduction observed slightly higher in the brushed regions (Table 3). Overall, the antimicrobial dentifrices reduced plaque 18 and 24%, respectively, for the shielded and brushed sites. As was the case for gingivitis, there was no apparent difference between the magnitude of anti-plaque benefit provided by the experimental dentifrices and that achieved with the stannous fluoride dentifrice.

Under clinical examination, there were no significant differences in the oral health status of the subjects across treatment groups. In response to a direct question regarding subject discomfort, there was no apparent difference in subject experience across treatment groups:

% Subjects Reporting Any Discomfort on Questionnaire

Negative Control	20%
Stannous fluoride dentifrice	24%
Experimental Dentifrice A	21%
Experimental Dentifrice B	11%

Consistent with their observed anti-plaque and anti-gingivitis efficacy, the stannous fluoride and 2 experimental dentifrices were also found to significantly ($p<0.05$) reduce, when compared to the negative control, the presence of gram-negative anaerobic (GNA) bacteria along the gingival margin (gum line) (Table 4). The stannous fluoride dentifrice and Experimental Dentifrice B also significantly reduced total anaerobic bacteria present along the gum line.

Summary

Several conclusions can be made from this study.

First, the 21-day, partial-mouth (tooth shield) experimental gingivitis model appears to have utility in evaluating dentifrices for anti-gingivitis and anti-plaque efficacy. The positive control stannous fluoride dentifrice was effective in this model with both gingivitis and plaque outcomes cleanly separating from the negative control. The gingivitis efficacy for the stannous fluoride dentifrice in this study also neatly mimicked the gingivitis outcomes observed for this product in more traditional, 6-month clinical trials. Interestingly, there is a difference in the plaque results obtained in this study versus those previously reported for a stannous fluoride dentifrice, with greater plaque reductions achieved in this study versus reductions reported in the longer term studies. This difference will be discussed later.

Gingivitis Efficacy for Stannous Fluoride Dentifrice Across Clinical Models
(% reduction vs. control, covariant adjusted)

		6-month clinical studies	
Indice	Current Study	1	2
GI	27	19	21
Gingival Bleeding	33	31	33
Plaque	20	3	3

1=Beiswanger *et al*, J. Clin. Denistry (1995), 6 (Special Issue), 46-53
2= Perlich *et al.*, J. Clin. Denistry (1995), 6 (Special Issue), 54-58

Overall, the efficacy results obtained in the toothshield EG model support using this model for assessing the effects of excipients on dentifrice efficacy.

Second, results from the study show that a stannous fluoride-containing dentifrice can provide clinically significant anti-plaque benefits in addition to its anti-gingivitis efficacy. As noted above, the stannous fluoride dentifrice provided in the current study provided significant plaque reductions in **both** the shielded and brushed regions, whereas in the cited 2 long-term studies lower plaque reductions were observed.

In the long term studies the authors speculated that their failure to observe a significant plaque reduction commensurate with the gingivitis efficacy of the tested product was due to interference with clinical plaque grading by confounding factors which developed over time (e.g. pellicle thickening, extrinsic staining). The results from this study indirectly support this hypothesis as the proposed confounding factors would not be much of an issue in the toothshield model since it is much shorter in duration.

Supporting the contention that 0.454% stannous fluoride in a dentifrice reduces plaque is the observation in this study that, coincident with its plaque reduction, treatment with this dentifrice caused significant reductions in the numbers of certain broad classes of bacteria residing along the gingival margin. As bacteria are a major component of plaque, this independent, objective measure clearly supports the proposition that Stannous fluoride dentifrice provides anti-plaque benefits.

Study No. 03PGC-H2

TABLE 1
ANALYSIS OF COVARIANCE[a]
DAY 21 GBI AND MGI (GINGIVITIS)
AVERAGE OF SHIELDED SITES
ALL SUBJECTS COMPLETING THE STUDY (N=150)

TREATMENT	N	ADJUSTED MEAN (SE)	FINAL % BENEFIT[b]	CHANGE % BENEFIT[c]	TREATMENT COMPARISON P-VALUES[d]		
					EXPERIMENTAL DENTIFRICE A	EXPERIMENTAL DENTIFRICE B	POSITIVE CONTROL
GINGIVAL BLEEDING INDEX (GBI BASELINE MEAN =0.16, ERROR VARIANCE=0.0902)							
Negative Control	39	0.51 (0.050)			0.0272	0.0153	0.0025
Experimental Dentifrice A	38	0.37 (0.050)	27	40		0.7891	0.2858
Experimental Dentifrice B	35	0.35 (0.052)	31	46			0.4222
Positive Control	38	0.29 (0.054)	43	63			
MODIFIED GINGIVAL INDEX (MGI BASELINE MEAN =1.04, ERROR VARIANCE=0.1300)							
Negative Control	39	1.72 (0.058)			0.0015	0.0805	<0.0001
Experimental Dentifrice A	38	1.47 (0.059)	15	37		0.1299	0.1316
Experimental Dentifrice B	35	1.60 (0.061)	7	18			0.0031
Positive Control	38	1.34 (0.059)	22	56			

[a] See Statistical Report for model details.

[b] Percent benefit compared to Negative Control calculated on the final adjusted mean. For example, the Positive Control % benefit is 100*(0.51-0.29)/0.51=43%.

[c] Percent benefit compared to Negative Control calculated on the adjusted mean *change from Baseline*. For example, the Positive Control benefit is 100*[(0.51-0.16)-(0.29-0.16)]/(0.51-0.16)=63%.

[d] *P*-values for comparisons involving the Negative Control are one-sided in the direction of greater efficacy for the other treatment. The remaining *p*-values are two-sided.

Study No. 03PGC-H2

TABLE 2
ANALYSIS OF COVARIANCE[a]
DAY 21 LÖE-SILNESS GINGIVAL INDEX
ALL SUBJECTS PRESENT[b] (N=143)

TREATMENT	N	ADJUSTED MEAN (SE)	% BENEFIT[c]	TREATMENT COMPARISON P-VALUES[d]		
				EXPERIMENTAL DENTIFRICE A	EXPERIMENTAL DENTIFRICE B	POSITIVE CONTROL
AVERAGE OF SHIELDED SITES (ERROR VARIANCE=0.1033)						
Negative Control	38	1.11 (0.053)		0.0515	0.0002	<0.0001
Experimental Dentifrice A	36	0.99 (0.054)	11		0.0634	0.0026
Experimental Dentifrice B	35	0.85 (0.055)	23			0.1820
Positive Control	34	0.73 (0.061)	34			
AVERAGE OF BRUSHED SITES (ERROR VARIANCE=0.0529)						
Negative Control	38	0.61 (0.037)		0.2496	0.0045	0.1051
Experimental Dentifrice A	36	0.57 (0.039)	7		0.0582	0.5644
Experimental Dentifrice B	35	0.46 (0.039)	25			0.1856
Positive Control	34	0.54 (0.039)	11			

[a] See Statistical Report for model details.

[b] The examiner was not available to examine all subjects.

[c] Percent benefit compared to Negative Control. For example, the Positive Control benefit is 100*(1.11-0.73)/1.11=34%.

[d] *P*-values for comparisons involving the Negative Control are one-sided in the direction of greater efficacy for the other treatment. The remaining *p*-values are two-sided.

Study No. 03PGC-H2

TABLE 3
ANALYSIS OF COVARIANCE[a]
MQH (PLAQUE)
AVERAGE OF SHIELDED SITES[b] AND BRUSHED SITES[c]
ALL SUBJECTS COMPLETING THE STUDY (N=150)

TREATMENT	N	ADJUSTED MEAN (SE)	% BENEFIT[d]	TREATMENT COMPARISON P-VALUES[e]		
				EXPERIMENTAL DENTIFRICE A	EXPERIMENTAL DENTIFRICE B	POSITIVE CONTROL
SHIELDED SITES - MODIFIED QUIGLEY-HEIN PLAQUE INDEX (MQH BASELINE MEAN=2.85, ERROR VARIANCE=0.1745						
Negative Control	39	3.75 (0.067)		<0.0001	<0.0001	<0.0001
Experimental Dentifrice A	38	3.14 (0.068)	16		0.3683	0.2445
Experimental Dentifrice B	35	3.05 (0.071)	19			0.8116
Positive Control	38	3.03 (0.068)	19			
BRUSHED SITES - MODIFIED QUIGLEY-HEIN PLAQUE INDEX (MQH BASELINE MEAN=2.85, ERROR VARIANCE=0.1536)						
Negative Control	39	2.69 (0.063)		<0.0001	<0.0001	<0.0001
Experimental Dentifrice A	38	1.99 (0.064)	26		0.2151	0.3772
Experimental Dentifrice B	35	2.10 (0.066)	22			0.7000
Positive Control	38	2.07 (0.064)	23			

[a] See Statistical Report for model details.

[b] Average of mandibular sites in the quadrant shielded during brushing.

[c] Average of mandibular sites in the quadrant not shielded during brushing

[d] Percent benefit compared to Negative Control calculated on the final adjusted mean. For example, the Positive Control % benefit is 100*(3.75-3.03)/3.75 =19%.

[e] *P*-values for comparisons involving the Negative Control are one-sided in the direction of greater efficacy for the other treatment. The remaining *p*-values are two-sided.

Study No. 03PGC-H2

TABLE 4
ANALYSIS OF COVARIANCE[a]
DAY 21 COLONY FORMING UNITS (LOG_{10} CFU/mL) ALONG MAXILLARY GUMLINE
ALL SUBJECTS WITH DATA (N=103)

TREATMENT	N	ADJUSTED MEAN (SE)	TREATMENT COMPARISON P-VALUES[b]		
			EXPERIMENTAL DENTIFRICE A	EXPERIMENTAL DENTIFRICE B	POSITIVE CONTROL
TOTAL FACULTATIVE ANAEROBES (LOG_{10} CFU/mL) (ERROR VARIANCE=0.1386) – ETSA MEDIA					
Negative Control	26	6.79 (0.073)	0.0587	0.0056	<0.0001
Experimental Dentifrice A	28	6.63 (0.070)		0.2813	0.0066
Experimental Dentifrice B	24	6.51 (0.076)			0.1102
Positive Control	25	6.34 (0.075)			
GRAM-NEGATIVE ANAEROBES (GNA) (LOG_{10} CFU/mL) (ERROR VARIANCE=0.1386) – ETSA NV MEDIA					
Negative Control	26	5.82 (0.107)	0.0002	<0.0001	0.0001
Experimental Dentifrice A	27	5.28 (0.105)		0.1868	0.7764
Experimental Dentifrice B	23	5.08 (0.113)			0.3048
Positive Control	25	5.24 (0.109)			

[a] See Statistical Report for model details.

[b] *P*-values for comparisons involving the Negative Control are one-sided in the direction of greater efficacy for the other treatment. The remaining *p*-values are two-sided.